PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2M.2AW46.61	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/002553	International filing date *(day/month/year)* 08.03.2004	Priority date *(day/month/year)* 10.06.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/70		
Applicant BIOM RIEUX B.V. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 12 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand 10.01.2005	Date of completion of this report 10.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Knehr, M Telephone No. +31 70 340-

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International application No.
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Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-58 as originally filed

Claims, Numbers

1-21 as originally filed

Drawings, Sheets

1/32-32/32 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 13

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 13

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☒ See separate sheet for further details

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☒ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
   - ☐ complied with.
   - ☒ not complied with for the following reasons:

     see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-21 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	11,12,15,18-21
	No:	Claims	1-4, 14,16,17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-4,11,12,14-21
Industrial applicability (IA)	Yes:	Claims	1-4,11,12,14-21
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

The following documents are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: ZHOU D-J ET AL.: "One-step duplex RT-PCR assay for detection SARS associated coronavirus" VIROLOGICA SINICA, vol. 18, no. 3, June 2003 (2003-06), pages 232-236

D2: JIE Y ET AL.: "Clinical detection of polymerase gene of SARS-associated coronavirus" ACADEMIC JOURNAL OF THE FIRST MEDICAL COLLEGE OF PLA, vol. 23, no. 5, May 2003 (2003-05), pages 424-427

D3: DROSTEN C ET AL: "IDENTIFICATION OF A NOVEL CORONAVIRUS IN PATIENTS WITH SEVERE ACUTE RESPIRATORY SYNDROME" NEW ENGLAND JOURNAL OF MEDICINE, MASSACHUSETTS MEDICAL SOCIETY, BOSTON, MA, US, vol. 348, no. 20, 15 May 2003 (2003-05-15), pages 1967-1976

D4: SHI R ET AL: "DESIGN AND APPLICATION OF 60MER OLIGONUCLEOTIDE MICROARRAY IN SARS CORONAVIRUS DETECTION" CHINESE SCIENCE BULLETIN, vol.48, no.12, June 2003, pages 1165-1169

D5: DEIMAN B ET AL: "CHARACTERISTICS AND APPLICATIONS OF NUCLEIC ACID SEQUENCE-BASED AMPLIFICATION (NASBA)" MOLECULAR BIOTECHNOLOGY, TOTOWA, NJ, US, vol. 20, no. 2, February 2002 (2002-02), pages 163-179

D6: TÄPP I ET AL: "Homogeneous scoring of single-nucleotide polymorphisms: Comparison of the 5'-nuclease TagMan assay and molecular beacon probes" BIOTECHNIQUES, vol. 28, no.4, 2000, pages 732-737

III. Non-establishment of opinion (Continuation)

III.1 As explained in the International Search Report, claim 13 does not meet the requirements of Article 6 PCT and has not been subject of a search. Therefore, claim 13 will not be examined (Rule 66.1(e) PCT).

IV. Lack of unity (Continuation)

IV.1 The Examining Division considers that there are 3 inventions covered by the claims indicated as follows:

I: Claims 1, 2, 11-21 (partially); 3, 4 (complete), directed to a pair of oligonucleotides for use as a set in the amplification of a target sequence located within the replicase gene of the genome of the SARS coronavirus, said pair consisting of fragments of at least 10 nucleotides of a first oligonucleotide according to SEQ ID NOS:1,3-5,9-11, in combination with a second oligonucleotide according to SEQ ID NOS:2,6-8; a (detectably labelled) oligonucleotide for use as a probe to detect the resulting amplified sequence, said probe comprising at least 10 nucleotides of SEQ ID NOS:12,13; the use of such an oligonucleotide pair as primers or probes within a method for detecting SARS nucleic acids; as well as a test kit, suitable for the detection of the SARS coronavirus in a sample, making use of such a primer pair or probe.

II: Claims 1, 2, 11-21 (partially); 5-8 (complete), directed to a pair of oligonucleotides for use as a set in the amplification of a target sequence located within the gene encoding the Nucleocapsid protein of the genome of the SARS coronavirus, said pair consisting of fragments of at least 10 nucleotides of a first oligonucleotide according to SEQ ID NOS:14-16,23-25,39-42, in combination with a second oligonucleotide according to SEQ ID ID NOS:17-20,26-29; a (detectably labelled) oligonucleotide for use as a probe to detect the resulting amplified sequence, said probe comprising at least 10 nucleotides of SEQ ID NOS:21,22,30; the use of such an oligonucleotide pair as primers or probes within a method for detecting SARS nucleic acids; as well as a test kit, suitable for the detection of the SARS coronavirus in a sample, making use of such a primer pair or probe.

III: Claims 1, 2, 11-21 (partially); 9, 10 (complete), directed to a pair of oligonucleotides for use as a set in the amplification of a target sequence located within the 3'-non coding region (3'-NCR) of the genome of the SARS coronavirus, said pair consisting of fragments of at least 10 nucleotides of a first oligonucleotide according to SEQ ID NOS:31-33,43,44, in combination with a second oligonucleotide according to SEQ ID NOS:34-37; a (detectably labelled) oligonucleotide for use as a probe to detect the resulting amplified sequence, said probe comprising at least 10 nucleotides of SEQ ID NOS:38,45,47; the use

of such an oligonucleotide pair as primers or probes within a method for detecting SARS nucleic acids; as well as a test kit, suitable for the detection of the SARS coronavirus in a sample, making use of such a primer pair or probe.

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

IV.2 Oligonucleotides used as amplification primers or detection probes for the specific detection of SARS nucleic acids within patient's samples are known from the prior art. Zhou et al., Virologica Sinica, vol.18(3), pp. 232-236 (June 2003) [D1] discloses the detection of SARS-Coronavirus by a method of PCR amplification, using primers corresponding with SEQ ID NOS:1,2,4-7, as claimed. Further on, Drosten et al., New Engl.J.Med., vol.348(20), pp. 1967-1976 (15-05-2003) [D3] discloses different methods of applying the polymerase chain reaction (e.g. nested PCR or real-time PCR) for that purpose, making use of SARS-specific primers hybridizing to the '1b region' encoding the SARS replicase gene, as well as detection of the amplified products using fluorophore-labelled hybridization probes (see especially pages 1969 an 1971, as well as tab.1). Likewise, Ksiazek et al., New Engl.J.Med., vol.348(20), pp. 1953-1955 (15-05-2003) [D5] discloses the molecular analysis of SARS using specific primers within an amplification method, including fluorophore-labelled primer (whole document).

IV.3 In view of the prior art, the problem of the underlying application can be defined as the provision of further SARS-specific oligonucleotides, suitable as primers and/or detection probes for amplifying and detecting specifically SARS-related nucleic acids within clinical probes by amplification in combination with detection by hybridization.

IV.4 Each of the defined 3 sets of primers and probes represent an independent solution concerning the underlying problem. Solution 1 is the provision of alternative primers and probes hybridizing to the part of the SARS genome encoding the replicase protein. Solution 2 is the provision of primers and probes hybridizing to the part of the SARS genome encoding the nucleocapsid protein. And solution 3 is the provision of primers and probes hybridizing to the part of the SARS genome representing the 3'-non-coding region.

IV.5 In view of the fact that primers and probes for amplification and detection of SARS-specific nucleic acids are already disclosed within the prior art (and explicitly oligonucleotides targeting the replicase region of SARS), due to essential differences in primary structure of the oligonucleotides claimed, and due to the fact that no other technical feature(s) could be identified which, in the light of the prior art, could be regarded as special technical features common to these solutions, in conclusion, the groups of claims are not linked by common or corresponding special technical features and define 3 different inventions not linked by a single general inventive concept.
The application, hence does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

V. Reasoned statement (Continuation)

V.1 NOVELTY (Art. 33(2) PCT)

V.1.1 D1 discloses a RT-PCR assay for the detection of SARS-associated coronavirus, specifically using primer pairs consisting of SEQ ID NOS:6 and 7, in combination with primers consisting of (shortened) complementary sequences of SEQ ID NOS: 4 and 5. D1 further discloses an amplicon comprising the entire sequence of probe SEQ ID NO:12 as claimed, as well as parts of probe SEQ ID NO:13 (abstract; Fig.'s 2 and 3; Tab.'s 1 and 2). In view of D1, claims 1-4, 16 and 17, are not novel over the prior art.

V.1.2 D2 discloses a PCR method for the clinical detection of the polymerase gene of SARS-associated coronavirus. Likewise to D1, D2 discloses primers consisting of SEQ ID NOS:6 and 7, as well as primers consisting of (shortened) complementary sequences of SEQ ID NOS: 4 and 5 (abstract; page 425, col.1, paragr.3; Fig.1). Also in view of D2, claims 1-4, 16 and 17, are not novel over the prior art.

V.1.3 Finally, D3 discloses a method of real-time nested PCR for the detection of the SARS virus including a double fluorescence-labelled probe comprising nucleotides 3-26 of SEQ ID NO:12 as claimed. The primers used in D3 for amplification derive from the very

same replicase 1b region of the SARS genome as the primers claimed within the application, however, not being identical ti the primer sequences as claimed (abstract; page 1969, col.1, last paragr. - col.2, paragr.2; page 1971, col.2, last paragr. - page 1972, col.1, paragr.2; Fig.2; Tab.'s 1 and 2). In view of D3, claim 14 is not novel over the prior art.

V.1.4 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-4, 14, 16 and 17, is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

V.1.5 However, the subject-matter of claims 5-11, 12, 15 and 18-21, can be considered to be new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

V.2 INVENTIVE STEP (Art. 33(3) PCT)

V.2.1 Document D3 is considered to represent the most relevant state of the art and discloses an amplification method (nested PCR) in combination with a double-fluorescence-labelled SARS-specific probe for detection of SARS-related coronavirus (the whole document). The difference between D3 and independent claims 5, 7, 9, 12, and 20 (product claims) as well as dependent claims 6, 8, 10 and 21, comprises specified and defined primer pairs/probes/kits as claimed. The difference between D3 and dependent claims 18 and 19 lies in the use of such primer pairs/probes within a method of detection by amplification. As compared to the prior art (D3), no specific technical seems to be related with the primer pairs/probes/kits as claimed as well as their use.

V.2.2 The problem to be solved by the subject matter of claims 5-12, 15, 18-20, and 21, can therefore be defined as the need of further primers and probes, suitable for the detection of SARS-associated coronavirus within a method of amplification, as well as kits comprising such primers/probes, suitable to carry out such a method of detection. The solution are the primer pairs as claimed (claims 5-12), probes as claimed (claim 15), kits comprising such primers and/or probes (claims 20-21), as well as methods of amplification

using them (claim 18-19), preferably within the NASBA methodology using primers comprising RNA promoter sequences, as well within TaqMan (real-time) detection assays using fluorescently-labelled (molecular beacon) probes.

V.2.3 However, this cannot be seen as comprising an inventive step for the following reasons:

V.2.4 Being aware of the teaching of especially D3, it would be obvious to the skilled person how to detect SARS-coronavirus using primers and probes within a detection method based on amplification of target sequences. Since documents D1 and D2 disclose some of the primers/probes as claimed, it would be obvious how to design and use oligonucleotides according (part of) the claims. Neither D3 nor other documents disclose the specific oligonucleotides as claimed within claims 5-12, and 15, however, the general principle how to design primers by choosing appropriate target sequences is well known to the person skilled in the art. Without any technical affect distinguishing the oligonucleotide sequences as claimed from others disclosed within the prior art, they must be seen as possible alternatives, e.g. those disclosed within D4 (Table 1), representing SEQ ID NOS:24 and 15, as claimed (amongst others). Therefore, claims 5-10 lack an inventive step over the disclosure of document D3 as combined with documents D1, D2 or D4.

V.2.5 Likewise, primers as claimed within claims 11 and 12 (comprising RNA promoter sequences at the 5'-end) being especially suitable within a NASBA amplification method, cannot be seen as inventive, since the principle of NASBA is well known from the prior art (e.g. D5), and the use of the NASBA within the context of the application is only one of several possibilities of amplifying well known from the prior art. Therefore, claims 11, 12, 18 and 19, lack an inventive step over the disclosure of document D3 as combined with document D5.

V.2.6 The same is valid in regards of using molecular beacon probes within a real-time TaqMan assay. It is without saying that such a detection method can be used in a general manner for any detection of nucleic acid targets. It is known from the prior art how to design and label molecular beacon probes, suitable for the detection of any specific gene of interest (e.g. from D6). Therefore, claim 15 lacks an inventive step over the disclosure of document D3 as combined with document D6.

V.2.7 The test kits of claims 20 and 21 comprising a set of primers as claimed as well as labelled detection probes as claimed are also not considered inventive, since the packaging of non-inventive subject-matter into a kit would be obvious to the skilled person. Thus, claim 20 as well as dependent claim 21 also do not satisfy the criterion set forth in Article 33(3) PCT, and the subject-matter of these claims does not involve an inventive step (Rule 65(1)(2) PCT).

V.2.8 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT since the subject-matter of claims 1-12, and 14-21, does not involve an inventive step as set forth in Rule 65(1)(2) PCT.

VI. Certain documents cited (Continuation)

VI.1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in documents D1, D2 and D5, is not mentioned in the description, nor are these documents identified therein.

VIII. Certain observations in the international application (Clarity) (Continuation)

VIII.1 The expression 'substantially' in claim 20 has no limiting effect on the scope of the claim and should be deleted.